https://doi.org/10.26651/allelo.j/2018-43-2-1147 0971-4693/94 Euro 20.00
Allelopathy Journal 43 (2): 275-288 (March, 2018) *International Allelopathy Foundation* 2018
Table: 2, Figs : 5

Effects of plant growth-promoting *Stremptomyces sampsonii* KJ40 on germination, seedling growth and enzymatic activities of maize

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(Received in revised form: January 23, 2018)

ABSTRACT

We separated and purified the plant growth-promoting bioactive compounds from the fermentation filtrate of *Stremptomyces sampsonii* KJ40 to investigate its effects on the growth of maize. Column chromatography and high performance preparative chromatography (HPPC) were used to obtain the monomer compound 16-M4. Its structure was identified using nuclear magnetic resonance (NMR) and electrospray ionization mass spectrometry (ESI-MS). In greenhouse experiments, the effects of different concentrations of bioactive compounds was determined on seed germination, biomass, photosynthesis characteristics and the identification of lipid peroxidation and related resistant enzymes activities (malondialdehyde (MDA), superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT)) of maize seedling. The results showed that the monomer compound 16-M4 was identified as cyclo- (Val-Pro). Inoculation of maize seeds with cyclo- (Val-Pro) (150 µg L^{-1}), significantly increased the germination index and vigour of maize seed and root length by 76.03%, 73.64%, and 54.52%, respectively. Besides, the photosynthesis, chlorophyll (Chl) content and net photosynthetic rates were increased by 27.97% and 103.81% than control. Results of this study suggested that the cyclo- (Val-Pro) had positive effects on seed germination and seedling growth of maize.

Key words: Bioassay, enzyme activity, germination, growth-promoting, maize, promotion, seedling growth, *Stremptomyces sampsonii* KJ40, *Zea mays*

INTRODUCTION

Maize (*Zea mays* L.), also called corn (45), is important food crop. Its nutritional value, accessibility and affordability have made it staple food worldwide. In 2014, its total world production was 1.04 billion tonnes, with United States and China providing 35% and 21% production, respectively (23). In addition, maize is also used to produce ethanol, corn starch, corn syrup and animal feed (3,6,12).

Maize is widely cultivated worldwide, but production is insufficient to meet the demand for consumption (33). The crop yield is affected by the available cultivars and series of pests and pathogens, such as corn borer (27), corn rootworm (37), rust, downy mildew (39) and stewart's wilt (8,10). To combat attacks from pests and pathogens, chemical pesticides and fertilizers are often used to protect crops and increase yield. However, treated with chemicals often creates new problems related to the sustainable development of agriculture, such as the efficacy of chemical treatments, environmental pollution, a disruption in

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biodiversity, insect resistance to pesticides, a lack of natural predators, and outbreaks of secondary pests (7,47). Hence, new biological pesticides and fertilizers have only added to concerns related to crop protection, production and quality.

Among the biological agents, actinomycetes promotes the plant growth as plant growth-promoting rhizobacteria (PGPR). Many researchers have identified some strains of actinomycetes, which not only prevented the insect pests and plant pathogens attack on crops, but also promoted the plant growth (4,11,28,34,47). Some actinomycetes or their secondary metabolites promoted the seed germination and roots growth, flowering and fruiting (9,14,24). The potential of actinomycetes have been explored for growth promotion and biocontrol of pests in rice, pea, tomato, wheat, bean (5,17,26,35,36,43,44).

The strain of *S. sampsonii* KJ40 was isolated from the poplar rhizosphere. Its antifungal protein effectively controls the *Rhizoctonia violacea* Tul & C. Tul, which causes poplar purple root rot (29) and also promotes plant growth. This study aimed to isolate and purify the plant growth promoting bioactive compounds from the fermentation filtrate of KJ40 and investigate their effects on the growth of maize plant in greenhouse.

MATERIALS AND METHODS

We obtained a sample of *S. sampsonii* KJ40 from the Laboratory of Forestry Protection in our University, Chengdu (30º7′N, 103º86′E, altitude: 535 m, annual precipitation: 918 mm, annual mean maximum and minimum temperatures: 26 to 13°C), Sichuan Province, China. The separation and purification assays were done from July to December 2016. Greenhouse experiments were done in July and from 2-4 August 2017, gas exchange, Chl content, MDA content and antioxidant enzyme were measured. Analytical grade chloroform, methanol, dichloromethane and organic reagents were purchased from Chengdu Kelong Chemical Co. Ltd. Chengdu. Chromatographic pure methanol used in chromatography was purchased from Sigma Reagent Co. Ltd. (St. Louis, MO, USA). Both silica gel GF254 used in thin-layer chromatography and silica gel of 60-80 or 200-300 meshes used in column chromatography were bought from Qingdao Marine Chemical Plant. (Qingdao, Shandong, China). Seeds of maize were purchased from Jixiu Seed international Co. Ltd. The experimental sandy soil was dried for 8 h at 110^{o}C and sieved through 20 mesh screen. The active culture medium was peptone 10 g, beef extract 3 g, agar 15 g, NaCl 5 g and H_2O 1000 mL at pH 7.0. The fermentation medium used was sorbitol 25 g, carbamide 15 g, K_2HPO_4 3.6 g、$CaCO_3$ 3 g and H_2O 1000 mL, with pH 7.0.

Separation and Purification Assays

The spore suspension (10^8 cfu·mL^{-1} concentration) dose of 1% (V/V), was inoculated in an Erlenmeyer 300 mL flask filled with 100 mL fermentation medium. It was cultured in a shaking incubator (at 28 ± 1 ^{o}C, 140 rpm, 4 d), then centrifuged at 8000 rpm at 4 ^{o}C for 15 min and the pellet was discarded. The supernatant was filtered by filter membrane of 0.45 mm and then stored in refrigerator at 4 ^{o}C for further experiments.

Fermentation filtrate (23 L) was collected and extracted with the same volume of chloroform. The organic and aqueous phase were collected and distilled with the rotary evaporator (BC-R501, Beikai, China) at 40 ^{o}C to obtain 6.032 g crude extracts. For further purification the crude extracts were mixed with silica gel using a 60-80 mesh (15 g).

After straining the silica gel with 200-300 mesh (70 g), the silica gel column (5 × 30 cm) was balanced with a buffer solution (dichloromethane:methanol 30:1). The prepared silica gel mixture was placed on the column and the column was subjected to gradient elution (dichloromethane:methanol 30:1-1:1). When the pigment zone reached bottom, the eluent was collected in a tube. According to the thin-layer chromatography (chromogenic agent: iodine), the eluent with the same component were combined.

For the seed germination test, the eluent which promotes the plant growth was analyzed by HPLC with a NP7001C pump and a NU3001 190-700 nm UV-detector (Hanbang, China). Testing conditions were: chromatographic column (4.6 × 250 mm, 5 µm) (Ultimate XB-C18, Yuexu, China); Mobile phase: Methanol: water 40:60; Flow rate: 100 mL min^{-1}; Testing wavelength: 210 nm; Column temperature: 15 ℃; Sample size: 20 µL.

Tests were conducted by bioassays and the single symmetrical peak was collected by HPPC. The HPPC system was equipped with an ASP2004-100 axial compression column, a 500 mL preparation pump, and a UV3000 UV-detector (Tongheng, China). Preparation conditions included chromatographic column (80 × 500 mm, 10 µm) (Ultimate XB-C18, Yuexu), Mobile phase: methanol:water 25:75, Flow rate: 140 mL min^{-1}, Testing wavelength: 210 nm, column temperature: 15^{o}C.

The molecular weight of cyclo- (Val-Pro) was measured by Q-Tof Premier Mass Spectrometer (Waters, Milford, MA, USA) using the ESI-MS method and its structure was determined by Super Conducting Fourier NMR Spectrometer AV II-600 (Bruker Corp., Fällanden, Switzerland).

Bioassays

The greenhouse experiments were done from 12 July to 4 August 2017. Before sowing, maize (*Zea mays* var. *ceratina*) seeds were sterilized with 0.5% (w/v) potassium hypermanganate solution for 15 min, rinsed with distilled water thrice and 120 seeds were placed per 15-cm Petri dishes. Fifty mL of diluent of cyclo- (Val-Pro) (50, 100, 150, 200 µg L^{-1} concentration) was added to each dish and soaked for 6 h. The distilled water was used as control treatment. The petri plates were kept in greenhouse (natural light, temperature 31-33^{o}C and relative humidity 35-42%). The treatments were replicated thrice in complete randomized design.

After the treatments, the transparent plastic square containers (21.3 cm length, 8.5 cm width, 3.9 cm height) were filled with sandy soil to 3 cm depth. Every 40 seeds were put in these sandy soil containers, covered with sand and cultured in the incubator (light 14 h, dark 10 h, 26^{o}C and relative humidity: 95%). The dishes for the three replicates of each treatment were watered daily with 20 mL of either the corresponding diluent or distilled water 20 mL as controls until harvest was done 10 d later. When the seedlings emerge from the sand surface the seed germination data was recorded at 5 h intervals for 3 d. Then the Germination Index [(GI; Eq. (1)], Germination Vigor [(Eq. (2)] and the inhibition or

stimulation of germinated [(%; Eq. (3)] were calculated as under:

$$GI = \sum \left({}^{G_t}/_{D_t} \right)$$

Where, G_t : Number of seeds germinated and D_t : Time after sowing (h)

Germination Vigour = GI × Hypocotyl length (cm)

Inhibition (-) or stimulation (+) percentage (%) = [(Extracts-Control)/Control] ×100

Before harvesting, in 10-seedlings per treatment, we measured photosynthesis and biometric parameters of plants, (epicotyl and hypocotyl length, root length, and fresh weight. Random samples of roots and leaves) and for physiological analyses.

Photosynthetic assays

Photosynthetic assays were conducted at 0800-1100 h on July 29, 2017. The photosynthetic parameters : net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (G_s) and intercellular CO_2 concentration (C_i) were measured by spectroradiometer and LI-6800 photosynthesis instrument (Li-Cor, Inc., Lincoln, NE, USA). During the measurement, a healthy leaf blade (uniform colour and the same position) was chosen from one seedling. Three seedlings were measured in each dish as replicates.

Chlorophyll was extracted from fresh leaves (0.5 g) with acetone and ethanol (1:1, V/V, 10 mL) added until the leaves turned white. Then, the absorbance of the extracts was detected using a Visible Spectrometer at 663 nm and 645 nm (V-1100D, Mapada Instruments Co., Ltd., Shanghai, China) (21,30).

Lipid peroxidation

Lipid peroxidation was determined in terms of MDA content as per the method of Hodges (18). In each treatment, fresh leaf samples (0.1 g) were randomly harvested, 8 mL of 10% TCA was added and the leaves were ground; this liquid was then centrifuged at 4000 rpm for 10 min, the supernatant was decanted, and the pellet discarded. Two mL of 0.6% TBA and 2 mL of supernatant (or distilled water for the control) were mixed in test tubes, which were boiled with water bath (at 100^{o}C for 10 min, counting started when the mixture started boiling). It was cooled down and mixed with ice and then the absorbance was measured at 450 nm, 532 nm, 600 nm by Visible Spectrometer.

Antioxidant enzyme assays

The fresh leave samples were ground with cooled 0.5 mol·L^{-1} phosphate buffer using a mortar and pestle, centrifuged at 4^{o}C, 1000rpm for 20 min. The supernatant was prepared to measure SOD, CAT and POD activity. SOD activity was detected using the nitroblue tetrazolium photo-reduction method (24). The supernatant was transferred in four test tubes, respectively (two measuring tubes, two controls), with different dosage of 0.05 mol·L^{-1} phosphate buffer added using 130 mmol·L^{-1} Met solution, 750 μmol·L^{-1} nitroblue tetrazolium solution, 100 μmol·L^{-1} EDTA-Na_2, and 20 μmol·L^{-1} riboflavin solution. One control tube was allowed to react in darkness, while the others reacted under 4000 Lx illumination. Then, each tube was measured at 560 nm by Visible Spectrometer, while the tube in darkness was used as control. CAT activity was measured using the method described by Aebi (1). The supernatant (1 mL) was decanted and mixed with 1.5 mL of

phosphate buffered saline and 1 mL of distilled water (controls: mixed with 2.5 mL of distilled water, boiled 1h to stop the enzymes activity before detection) in the test tube. Next, 0.3 mL of H_2O_2 was added to each tube, then the value was recorded at 240 nm every 1 min lasting 4 min by Ultraviolet Spectrophotometer (Biomate 3S, Thermo Scientific, Waltham, MA, USA). POD activity of leaves was detected by the guaiacol method (20). First, 1mL of guaiacol (same volume of distilled water as control) was added to a test tube with leaf material, and then the supernatant was measured by the ultraviolet spectrophotometer at 470 nm for 4 min, with measurements taken every 1 min.

Statistical analysis: Experiments to evaluate seed germination and plant physiological status were done in randomized design, while the differences of data among treatments were tested by one-way analysis of variance. Treatment means were calculated by Duncan's multiple range test ($P < 0.05$). All statistical analyses were performed with SPSS 19.0 statistical software. Data are expressed as mean ± standard error.

RESULTS AND DISCUSSION

Structural analysis and identification of 16- M4

The bioactive substance of 16-M4 was a white powder, its chemical structure was confirmed by ESI-MS, ^{1}H-NMR, and ^{13}C-NMR. In ESI-MS, strong peaks were recorded at 197.16, 219.14 and 195.13, which correspond to $[M+H]^+$, $[M+Na]^+$, and $[M-H]^-$, respectively. The molecular weight of 16-M4 may be 196. In the ^{1}H-NMR spectrum (600MHz, methanol-d), two signals of methyl hydrogen δ_H were observed, 0.83(3H, d, J = 6.6Hz) and 0.99(3H, d, J = 6.6Hz), coupling in the methyne carbon in the high field section. In addition, there were other hydrogen single, such as δ_H 1.82 (2H, m), 1.92 (1H, m), 2.21 (1H, m), 2.39 (1H, m), 3.38 (1H, m), 3.45 (1H, m), 4.10 (1H, m), 4.50 (1H, m) towards the low field. In the ^{13}C-NMR spectrum (600MHz, methanol-d), the 2 carbonyl carbon signals at δ_C 171.1, 166.1 were speculated to be a functional group of carboxyl, ester functionalities or acid amides from their chemical shifts. Also, signals occurred at 60.1 and 58.6 ppm, the two carbons affected by the shielding effect moving to the low field are inferred to have coupling with N, while the other six carbons signals δ_C 44.7, 28.4, 28.1, 21.8, 17.4, and 15.2 in the high filed section included two signals of methyl carbon (δ_C 17.4, 15.2). A search using SciFinder found the results of NMR agreed with the finding of cyclo- (Val-Pro) that has been reported in earlier research (22). Therefore, 16-M4 was identified as cyclo- (Val-Pro) (Fig. 1).

Seed germination and seedling growth of maize

Seeds were soaked for 6 h in 15-cm Petri dishes that filled with diluent of cyclo- (Val-Pro) 50 mL (50, 100, 150, 200 µg L^{-1} concentration) and then cultured in an incubator, seeds began to germinate 23 h later (Fig. 2). In the first 20 h, seeds germinated rapidly, afterwards the percentage of newly germinated seeds increased slowly with time. The seeds treated with cyclo- (Val-Pro) at 150 µg L^{-1} concentration, germinated earlier than control seeds.

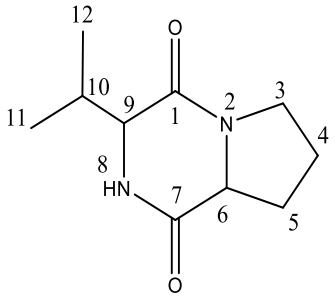

Figure 1. Two-dimensional structural formula of cyclo- (Val-Pro)

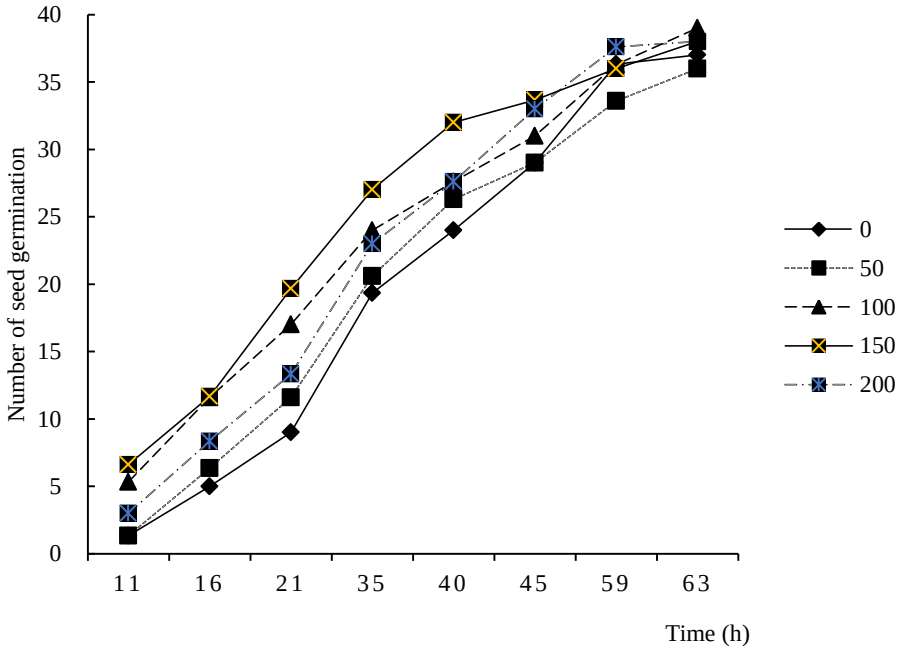

Figure 2. Effects of cyclo- (Val-Pro) concentrations of 0 (control), 50, 100, 150 and 200 μg L^{-1} on seeds germination

The seed germination (90.00-96.67) did not vary significantly between the treatments (Figure 3). The GI of the control was 1.19 and it increased with the increasing concentration of cyclo- (Val-Pro). At 150 μg L^{-1} concentration, the GI grew to 2.10, an increase of 76.47% than control. However, the GI decreased to 1.45 at the 200 μg L^{-1} concentration. Seeds vigour also exhibited similar increasing curve like the GI i.e. increased cyclo- (Val-Pro) concentration resulted in increased the vigour of maize seeds.

At 100 and 150 µg L^{-1} concentration of cyclo- (Val-Pro), seeds vigour significantly increased by 73.64% and 72.10% over the control. However, seeds vigour did not increase at the higher concentration of 200 µg L^{-1}.

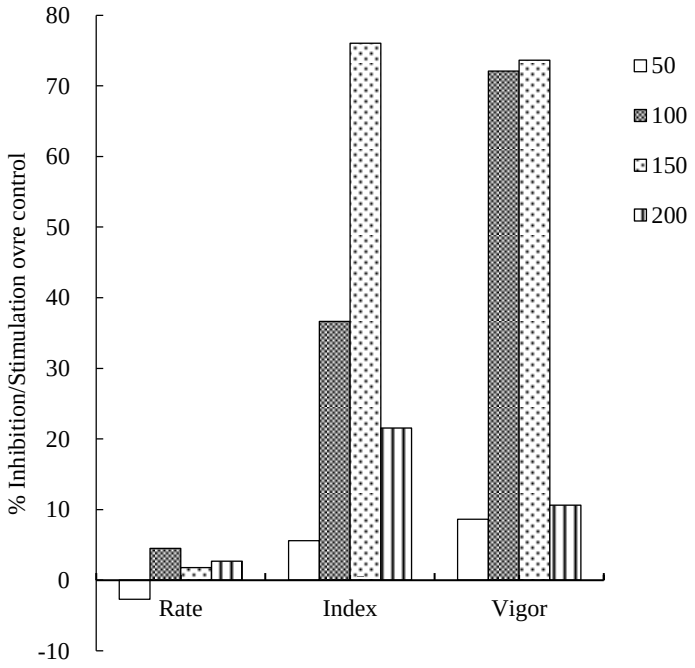

Figure 3. Inhibitory/stimulatory effects of cyclo- (Val-Pro) concentrations 0 (control), 50, 100, 150 and 200 µg L^{-1} on seed germination (%), germination index, and growth of maize plants
+: Stimulation, -: Inhibition.

The epicotyl, hypocotyl and root length were measured and compared with control (Table 1, Figure 4). Epicotyl length did not vary significantly between the treatments. When treated with 100 µg L^{-1} solution of cyclo- (Val-Pro), the hypocotyl length increased significantly to 8.93 cm compared with 7.07 cm in control.

Table 1. Effects of different concentrations of cyclo- (Val-Pro) from *Streptomuyces Sampsonii* on the seedling growth of maize seedlings

Concentrations (µg L^{-1})	Seedling length (cm)		Root:shoot ratio
	Epicotyl	Hypocotyl	
0	15.94±1.83a	7.07±0.54a	0.71±0.2a
50	17.98±2.33a	7.25±0.58a	0.91±0.36a
100	17.64±1.60a	8.93±0.83b	0.76±0.18a
150	16.81±4.66a	7.08±0.70a	0.67±0.12a
200	17.89±1.94a	6.56±0.84a	0.75±0.20a

The application of solution culture of cyclo- (Val-Pro) significantly increased the root length and leaf area (Figure 4). The 150 µg L^{-1} concentration of cyclo- (Val-Pro) solution increased the root length by 54.5% and the leaf area by 124.1% than control.

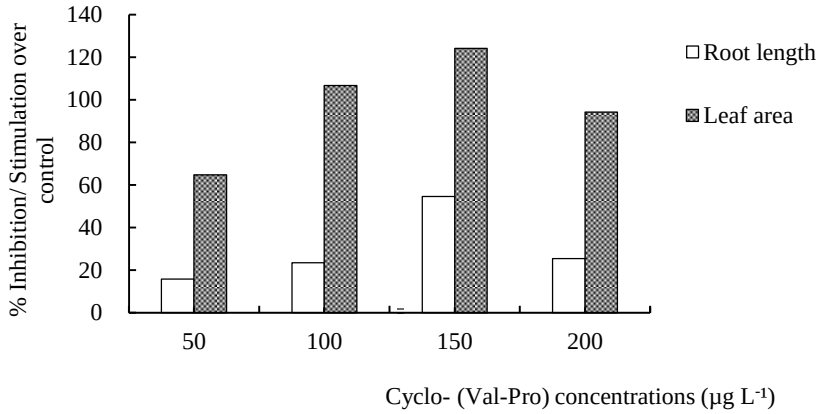

Figure 4 Inhibitory/stimulatory effects of cyclo- (Val-Pro) concentrations 0 (control), 50, 100, 150 and 200 µg L^{-1} on root length and leaf area of maize seedlings

+: Stimulation, -: Inhibition.

In a previous study, actinobacteria promoted the plant growth (seed germination, lengths of root and shoot as well as increased fresh and/or dry weight) (31, 34). The endophyte strain of *Streptomyces* sp. PT2 improved the seed germination and stimulated the root elongation in tomato (46). Similar morphological changes occurred in rice and mung bean plants, where root and shoot lengths increased with the strain *Streptomyces* sp. GMKU 3100 treatment (40). Likewise, *Streptomyces spiralis* Falcão de Morais increases the root and shoot length and dry weight of cucumber under commercial field production conditions (19). In the present experiment in greenhouse conditions, 150 µg L^{-1} of bioactive compound cyclo- (Val-Pro) stimulated the seed germination, enhanced the GI by 76.03% and seeds vigour by 73.64% than control ($P < 0.05$). In addition, the150 µg L^{-1} of cyclo- (Val-Pro) significantly increased the root length and leaf total area than control ($P < 0.05$). The results of our present study were consistent with those of earlier studies.

Chl content and photosynthesis

The cyclo- (Val-Pro) solution influenced the Chl content and gas exchange of the maize. In this experiment, Chl content, P_n, T_r, and G_S increased, while C_i decreased under all levels of cyclo- (Val-Pro) solution (Table 2). Under the treatments of 150 µg L^{-1} cyclo- (Val-Pro), significantly increased the Chl content by 27.97% in the maize seedlings. The P_n, T_r and G_S in plants inoculated with cyclo- (Val-Pro) solution were significantly higher than control. The maize seedlings treated with 150 µg L^{-1} cyclo- (Val-Pro) had the highest P_n, T_r and G_S of 6.95, 1.48 and 44.73, respectively. Compared with those of control plants, the P_n of seedlings inoculated with 150 µg L^{-1} cyclo- (Val-Pro) increased by 100.38%, however, T_r

and G_S were enhanced slightly. The C_i of control was 332.66 µmol mol^{-1}. The application of cyclo- (Val-Pro) solution clearly decreased the C_i of seedlings. Marius *et al* (41) reported similar results with *Bacillus pummilus* and *Bacillus mycoides* Flügge these promoted the photosynthesis, transpiration and leaves chlorophyll content in runner bean (*Phaseolus coccineus* L.). Additionally, Baset found that the photosynthetic rate of banana increased after co-inoculation with *Azospirillum brasilense* Tarrand, Krieg & Döbereiner (25).

Table 2. Effects of different concentrations of cyclo- (Val-Pro) from *Streptomuyces Sampsonii* on photosynthesis in maize leaves

Concentrations (µg L^{-1})	Chlorophyll content (g g^{-1}FW)	Net photosynthetic rate (P_n) (µmol m^{-2} s^{-1})	Transpiration rate (T_r) (mmol m^{-2} s^{-1})	Intercellular CO_2 concon (C_i) (µmol mol^{-1})	Stomatal conductance (G_S) (mmol m^{-2} s^{-1})
0	2.61±0.17a	3.41±3.50a	0.99±0.79a	332.66±196.57a	28.13±23.17a
50	2.90±0.13ab	6.71±0.41ab	1.29±0.06a	103.43±14.76b	39.68±1.84a
100	3.05±0.21bc	5.72±0.91ab	1.36±0.31a	151.86±26.16b	41.05±10.86a
150	3.34±0.10c	6.95±1.15b	1.48±0.69a	106.53±61.40b	44.73±21.55a
200	3.13±0.19bc	6.41±0.44ab	1.10±0.02a	81.03±24.37b	33.88±0.62a

Note: FW, fresh weight.

After the leaves emergence, the plant photosynthesis converts light energy into chemical forms to support various activities of organisms. P_n was mainly affected by Chl content, G_S, C_i and T_r. In previous studies. PGPR effectively promoted the photosynthesis in cabbage (48), banana (25) and black locust seedlings (49). In the assays of present study, cyclo- (Val-Pro) at a 150 µg L^{-1} concentration enhanced the Chl content and increased the P_n. However, the interaction between P_n, G_S, C_i and T_r still require further study.

Stimulation in physiology status of maize seedlings

When inoculated with cyclo- (Val-Pro), physiological status of plant changed; cyclo- (Val-Pro) affected the lipid peroxidation of plant tissue and the activity of defence enzymes (SOD, CAT, POD). Lipid peroxidation was reflected by the MDA content (Figure 5) which was promoted at 50-100 µg L^{-1} concentration of cyclo- (Val-Pro), while cyclo- (Val-Pro) in range of 150-200 µg L^{-1} inhibited the lipid peroxidation. The MDA content in plants inoculated with 100 µg L^{-1} of cyclo- (Val-Pro) increased by 139.75% than control. Similar to the MDA content, the activities of POD and CAT were also enhanced with 50 and 100 µg L^{-1} cyclo- (Val-Pro) solution, but decreased in the 150 and 200 µg L^{-1} treatments. Below 100 µg L^{-1} of cyclo- (Val-Pro) treatment the CAT activity was significantly increased (194.44%). The cyclo- (Val-Pro) treatment did not influence the SOD activity which regulates the promotion or inhibition of seedlings growth.

In earlier studies, inoculation with cyclo- (Val-Pro) enhanced the MDA content and the enzymes activities, that resist the oxidation. In this study, the MDA content increased with 150 µg L^{-1} concentration of cyclo- (Val-Pro). Similarly the MDA content increased, when rice was inoculated with the *Streptomyces* spp. (16). During oxidative damage to the plant tissue, superoxide (O^{2-}) can be converted into O_2 and hydrogen peroxide (H_2O_2) by SOD. CAT catalyses the breakdown of H_2O_2 and POD decomposes it by oxidation of substrates. The induction of *Streptomyces* spp. PM9 raised the SOD enzymes activity in

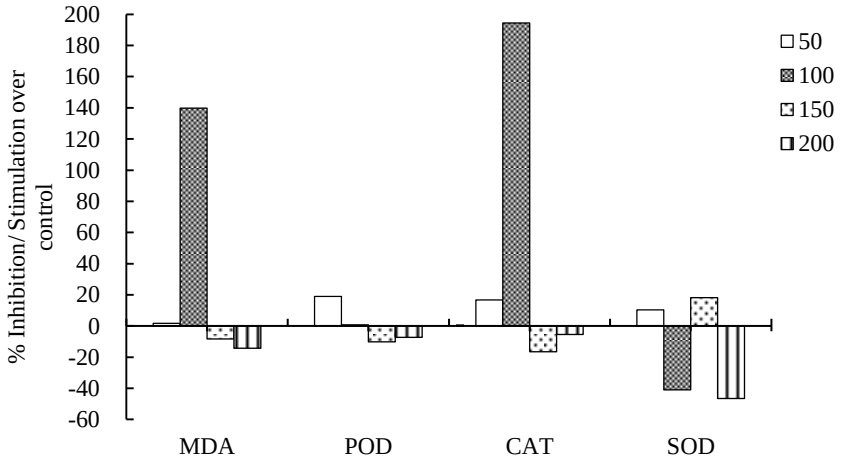

Figure 5. Inhibitory/stimulatory effects of cyclo- (Val-Pro) concentrations of 0 (control), 50, 100, 150 and 200 µg L^{-1} on activities of lipid on lipid peroxidation and major antioxidant enzymes [malondiadehyde (MDA), peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD)] of maize seedlings

+: Stimulation, -: Inhibition.

plants of *Eucalyptus grandis* W. Hill ex Maiden and *E. globulus* Labill (42). Similar phenomena have been reported in wheat (2), chickpea (15), *Vigna* spp. (31). In this regard, cyclo- (Val-Pro) had no effect on the activity of SOD enzymes. CAT and POD scavenge the H_2O_2, eliminate the lipid peroxide and protect the cell membranes against oxidation (8,13,26). Enzymatic activity increased with inoculation of PGPR, including the *Streptomyces* spp. KLBMP 5084 (32). Only CAT enzyme activity was significantly enhanced by the inoculation of 100 µg L^{-1} cyclo- (Val-Pro). The occurrence of these variations might be the reason for variable reactions of different plants to cyclo- (Val-Pro) stimulation.

CONCLUSIONS

Inoculation of the cyclo- (Val-Pro) at 150 µg L^{-1} promoted the seed germination, enhanced the biomass and Chl content and the net photosynthetic rate in maize plants. Further studies will analyse the effects of the cyclo- (Val-Pro) on maize at various development stages and evaluate its' effectiveness on maize plants in the field condition. The cyclo- (Val-Pro) has been separated from the *Stremptomyces sampsonii* KJ40. More natural substance will be explored from the *Stremptomyces* in the future.

ACKNOWLEDGEMENTS

Authors thank Dr. M. Hao, for providing technical assistance. This research was supported by the National Natural Science Foundation of China (No. 31070578).

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